

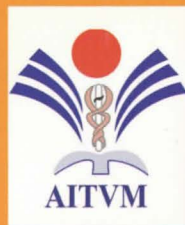
# Proceedings

## The 12th International Conference of THE ASSOCIATION OF INSTITUTIONS FOR TROPICAL VETERINARY MEDICINE

Does control  
of animal  
infectious  
risks offer  
a new  
international  
perspective ?



Montpellier, France  
20-22 August 2007



## **DEVELOPMENT OF A DIVA VACCINE AGAINST *PESTE DES PETITS RUMINANTS* BY REVERSE GENETIC**

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### **ABSTRACT**

Peste des Petits Ruminants (PPR) is a contagious viral disease of goats, sheep and wildlife in sub-Saharan African countries, Middle East and South-West of Asia. It is caused by a single strand negative RNA virus, belonging to the *Paramyxoviridae* family and *Morbillivirus* genus. Current vaccines consist of viral strains attenuated by several passages on cell cultures. These vaccines protect animals against PPR but do not permit the distinction between vaccinated and infected animals, a possibility which could improve surveillance and eradication of the disease. Development of reverse genetic to manipulate negative strand RNA genome is helping us to generate an infectious clone of PPR after cloning the whole genome of the PPR vaccine strain. Then, the objective is to mark this genome with a negative tag by changing B epitopes on the N gene and a positive mark by insertion of a tag between P and M genes. A double marked vaccine of PPR is necessary for the discrimination of vaccinated, infected or vaccinated and concomitantly infected animals. Development of appropriate companion diagnosis tests are also in progress.

### **INTRODUCTION**

Peste des Petits Ruminants (PPR) is a contagious viral disease of goats, sheep and wildlife in sub-Saharan African countries, Middle East and South-West of Asia. It is caused by a single strand negative RNA virus, belonging to the *Paramyxoviridae* family and *Morbillivirus* genus. Current vaccines consist of viral strains attenuated by several passages on cell cultures. These vaccines protect animals against PPR but do not permit the distinction between vaccinated and infected animals. Development of reverse genetic to manipulate negative strand RNA genome allows generating an infectious marked clone of PPR vaccine strain.

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## **MATERIALS AND METHODS**

A rescue system with a minigenome containing the eGFP reporter gene was first generated. This gene was placed between the leader and trailer of PPRV in antisens position between the T7 polymerase promoter and terminator. This minigenome was co-transfected on 293-T7 cells (293 cells which expressed the T7 RNA polymerase) with 3 plasmids containing the N, P and L gene of PPRV to reconstitute the viral ribonucleoprotein complex. Four clones covering the full-length genome of PPRV were assembled in antisens orientation between T7 promoter and terminator into a modified pBluescript. This full length genome was co-transfected as previously described for the minigenome. Cells were harvested three days later, co-cultured on Vero cells and passaged until CPE developed.

## **RESULTS**

The rescue system using the eGFP minigenome and 293-T7 cells generated fluorescent cells, 3 days after transfection. These results were confirmed by detection of eGFP mRNA in transfected cells. The full length genome of PPRV vaccine strain was assembled and the sequence checked (this sequence was deposited in GenBank n°X74443). Generation of the first infectious clone of PPRV by reverse genetic is ongoing.

## **DISCUSSION AND CONCLUSIONS**

The objective was to develop the reverse genetic for PPRV and to generate a first clone of PPRV. The rescue system based on the use of a minigenome expressing the eGFP was validated. Attempts to generate an infectious clone of PPRV from the full-length genome are ongoing. The final objective is to insert a mark in this genome and to develop appropriate companion diagnosis tests.

## **Acknowledgements**

The authors would like to thank F. Tangy (Institut Pasteur, Paris, France), T. Barrett (Institute of Animal Health, Pirbright, Great Britain) and M. Skinner (Institute of Animal Health, Compton, Great Britain). This study is partially granted by the UE Markvac project and EPIZONE network of excellence.

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